

CLAIM AMENDMENTS

Claim 1 (currently amended): A combination against dental caries bacteria, characteristic in comprising of effective components composed with IgY to dental caries bacteria and[[,]] at least[[,]] one of ~~both~~ potassium sorbate and sodium benzoate.

Claim 2 (currently amended): The combination, as recited in claim 1, wherein an additive amount of the IgY is at least ~~over 0.05% of additive IgY amount,~~ and additive amount of the potassium sorbate and the sodium benzoate is 0.005-0.02% respectively.

Claim 3 (currently amended): The combination, as recited in claim 2, wherein the additive amount of the IgY ~~amount~~ is 0.05-0.2%.

Claim 4 (currently amended): The combination, as recited in claim 3, wherein as the combination which is a liquid product used for oral cavity is packaged in pocket atomizer for spraying usage.

Claim 5 (currently amended): The combination, as recited in claim 3, wherein as the combination which is a liquid food ~~the combination~~ is packaged in sucking bottle.

Claim 6 (new): The combination, as recited in claim 1, wherein the IgY against dental caries bacteria is prepared by the following steps:

(a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;

(a1) separately cultivating the streptococcus mutans type c and type d in a culture medium for 2 to 3 days;

(a2) collecting bacteria by centrifugation;

(a3) washing the bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;

(a4) mixing the streptococcus mutans type c and type d in a ratio of 2:1; and

- (a5) adding Freund's adjuvant equal to total volume of the streptococcus mutans type c and type d with high speed homogenized;
- (b) immunizing hens with the streptococcus mutans antigen to obtain eggs with active antibody from the hens for 13 months;
- (c) extracting a crude IgY from the eggs by water dilution method;
- (d) applying the crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;
- (e) applying the eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;
- (f) collecting the new eluates of protein peak;
- (g) estimating antibody activity of the eluates of protein peaks with "ELISA";
and
- (h) eliminating bacteria by 0.22 μ m membrane filtration and lyophilizing to achieve the IgY against dental caries bacteria.

Claim 7 (new): The preparation method, as recited in claim 6, wherein the step (b) comprises the steps of:

- (b1) immunizing the hens by three hypodermic injections of 1×10^9 /ml of the streptococcus mutans antigens each time at two weeks intervals;
- (b2) collecting and sterilizing the eggs from 20th day after the first hypodermic injection; and
- (b3) taking out yolks from the eggs by sieve.

Claim 8 (new): The preparation method, as recited in claim 7, wherein the step (c) comprises the steps of:

(c1) evenly stirring the yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution;

(c2) adjusting the diluted yolk solution to pH 4.5-6.5;

(c3) standing the diluted yolk solution at 3-5°C for 20-30 hours;

(c4) centrifuging the diluted yolk solution for 20-30 minutes to obtain a supernatant; and

(c5) concentrating the supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve the crude IgY.

Claim 9 (new): The combination, as recited in claim 2, wherein the IgY against dental caries bacteria is prepared by the following steps:

(a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;

(a1) separately cultivating the streptococcus mutans type c and type d in a culture medium for 2 to 3 days;

(a2) collecting bacteria by centrifugation;

(a3) washing the bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;

(a4) mixing the streptococcus mutans type c and type d in a ratio of 2:1; and

(a5) adding Freund's adjuvant equal to total volume of the streptococcus mutans type c and type d with high speed homogenized;

(b) immunizing hens with the streptococcus mutans antigen to obtain eggs with active antibody from the hens for 13 months;

(c) extracting a crude IgY from the eggs by water dilution method;

(d) applying the crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;

(e) applying the eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;

(f) collecting the new eluates of protein peak;

(g) estimating antibody activity of the eluates of protein peaks with "ELISA";
and

(h) eliminating bacteria by 0.22µm membrane filtration and lyophilizing to achieve the IgY against dental caries bacteria.

Claim 10 (new): The preparation method, as recited in claim 9, wherein the step (b) comprises the steps of:

(b1) immunizing the hens by three hypodermic injections of 1×10^9 /ml of the streptococcus mutans antigens each time at two weeks intervals;

(b2) collecting and sterilizing the eggs from 20th day after the first hypodermic injection; and

(b3) taking out yolks from the eggs by sieve.

Claim 11 (new): The preparation method, as recited in claim 10, wherein the step (c) comprises the steps of:

(c1) evenly stirring the yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution;

(c2) adjusting the diluted yolk solution to pH 4.5-6.5;

(c3) standing the diluted yolk solution at 3-5°C for 20-30 hours;

(c4) centrifuging the diluted yolk solution for 20-30 minutes to obtain a supernatant; and

(c5) concentrating the supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve the crude IgY.

Claim 12 (new): The combination, as recited in claim 3, wherein the IgY against dental caries bacteria is prepared by the following steps:

(a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;

(a1) separately cultivating the streptococcus mutans type c and type d in a culture medium for 2 to 3 days;

(a2) collecting bacteria by centrifugation;

(a3) washing the bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;

(a4) mixing the streptococcus mutans type c and type d in a ratio of 2:1; and

(a5) adding Freund's adjuvant equal to total volume of the streptococcus mutans type c and type d with high speed homogenized;

(b) immunizing hens with the streptococcus mutans antigen to obtain eggs with active antibody from the hens for 13 months;

(c) extracting a crude IgY from the eggs by water dilution method;

(d) applying the crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;

(e) applying the eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;

(f) collecting the new eluates of protein peak;

(g) estimating antibody activity of the eluates of protein peaks with "ELISA";
and

(h) eliminating bacteria by 0.22µm membrane filtration and lyophilizing to achieve the IgY against dental caries bacteria.

Claim 13 (new): The preparation method, as recited in claim 12, wherein the step (b) comprises the steps of:

(b1) immunizing the hens by three hypodermic injections of 1×10^9 /ml of the streptococcus mutans antigens each time at two weeks intervals;

(b2) collecting and sterilizing the eggs from 20th day after the first hypodermic injection; and

(b3) taking out yolks from the eggs by sieve.

Claim 14 (new): The preparation method, as recited in claim 13, wherein the step (c) comprises the steps of:

(c1) evenly stirring the yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution;

(c2) adjusting the diluted yolk solution to pH 4.5-6.5;

(c3) standing the diluted yolk solution at 3-5°C for 20-30 hours;

(c4) centrifuging the diluted yolk solution for 20-30 minutes to obtain a supernatant; and

(c5) concentrating the supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve the crude IgY.

Claim 15 (new): The combination, as recited in claim 3, wherein the IgY against dental caries bacteria is prepared by the following steps:

(a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;

(a1) separately cultivating the streptococcus mutans type c and type d in a culture medium for 2 to 3 days;

- (a2) collecting bacteria by centrifugation;
- (a3) washing the bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;
- (a4) mixing the streptococcus mutans type c and type d in a ratio of 2:1; and
- (a5) adding Freund's adjuvant equal to total volume of the streptococcus mutans type c and type d with high speed homogenized;
- (b) immunizing hens with the streptococcus mutans antigen to obtain eggs with active antibody from the hens for 13 months;
- (c) extracting a crude IgY from the eggs by water dilution method;
- (d) applying the crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;
- (e) applying the eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;
- (f) collecting the new eluates of protein peak;
- (g) estimating antibody activity of the eluates of protein peaks with "ELISA"; and
- (h) eliminating bacteria by 0.22µm membrane filtration and lyophilizing to achieve the IgY against dental caries bacteria.

Claim 16 (new): The preparation method, as recited in claim 15, wherein the step (b) comprises the steps of:

- (b1) immunizing the hens by three hypodermic injections of 1×10^9 /ml of the streptococcus mutans antigens each time at two weeks intervals;

(b2) collecting and sterilizing the eggs from 20th day after the first hypodermic injection; and

(b3) taking out yolks from the eggs by sieve.

Claim 17 (new): The preparation method, as recited in claim 16, wherein the step (c) comprises the steps of:

(c1) evenly stirring the yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution;

(c2) adjusting the diluted yolk solution to pH 4.5-6.5;

(c3) standing the diluted yolk solution at 3-5°C for 20-30 hours;

(c4) centrifuging the diluted yolk solution for 20-30 minutes to obtain a supernatant; and

(c5) concentrating the supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve the crude IgY.

Claim 18 (new): The combination, as recited in claim 5, wherein the IgY against dental caries bacteria is prepared by the following steps:

(a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;

(a1) separately cultivating the streptococcus mutans type c and type d in a culture medium for 2 to 3 days;

(a2) collecting bacteria by centrifugation;

(a3) washing the bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;

(a4) mixing the streptococcus mutans type c and type d in a ratio of 2:1; and

- (a5) adding Freund's adjuvant equal to total volume of the streptococcus mutans type c and type d with high speed homogenized;
- (b) immunizing hens with the streptococcus mutans antigen to obtain eggs with active antibody from the hens for 13 months;
- (c) extracting a crude IgY from the eggs by water dilution method;
- (d) applying the crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;
- (e) applying the eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;
- (f) collecting the new eluates of protein peak;
- (g) estimating antibody activity of the eluates of protein peaks with "ELISA";
and
- (h) eliminating bacteria by 0.22µm membrane filtration and lyophilizing to achieve the IgY against dental caries bacteria.

Claim 19 (new): The preparation method, as recited in claim 18, wherein the step (b) comprises the steps of:

- (b1) immunizing the hens by three hypodermic injections of 1×10^9 /ml of the streptococcus mutans antigens each time at two weeks intervals;
- (b2) collecting and sterilizing the eggs from 20th day after the first hypodermic injection; and
- (b3) taking out yolks from the eggs by sieve.

Claim 20 (new): The preparation method, as recited in claim 19, wherein the step (c) comprises the steps of:

(c1) evenly stirring the yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution;

(c2) adjusting the diluted yolk solution to pH 4.5-6.5;

(c3) standing the diluted yolk solution at 3-5°C for 20-30 hours;

(c4) centrifuging the diluted yolk solution for 20-30 minutes to obtain a supernatant; and

(c5) concentrating the supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve the crude IgY.